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The Prognostic Value of Extracellular Matrix Component Concentrations in Serum During Treatment of Adult Respiratory Distress Syndrome with Extracorporeal CO_2 Removal

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Summary: The time-dependent concentrations of hyaluronan, aminoterminal propeptide of type III procollagen, and laminin were determined in sera of 16 patients with severe adult respiratory distress syndrome during treatment with an extracorporeal CO_2 removal device. Patients were classified according to lung parameters as responders (n = 10) and non-responders (n = 6) to extracorporeal CO_2 removal. At the beginning of treatment strongly elevated serum concentrations of all studied extracellular matrix components were found. During the first 6–11 days of treatment the concentrations of aminoterminal propeptide of type III procollagen and hyaluronan increased further in non-responders but decreased in the majority of responders, while laminin decreased in both groups. No significant correlations were found between the serum concentrations of connective tissue components and the parameters of lung function. By non-parametric analysis of variance, significant differences between responders and non-responders according to treatment time could be established. By analysing the time course of the serum concentrations of hyaluronan and aminoterminal propeptide of type III procollagen, a total differentiation between responders and non-responders was made possible by the trends of these analytes as early as three days after the start of treatment. The determination of aminoterminal propeptide of type III procollagen and hyaluronan in serum of patients with adult respiratory distress syndrome might therefore have prognostic significance in extracorporeal CO_2 removal.

Introduction

The adult respiratory distress syndrome is a severe condition of life-threatening organ failure, characterized by tachypnoea, hypoxaemia, diffuse interstitial infiltrates, alveolar oedema, and loss of lung compliance (1, 2). The syndrome develops in response to a primary attack on lung cells like inhalation of toxins, aspiration of gastric contents (3) or as a consequence of systemic disorders like septic shock and multiorgan trauma. Severe, progressive cases are associated with a mortality rate of up to 90% (4). One sequel of adult respiratory distress syndrome is the prognostically unfavourable rapid development of pulmonary fibrosis within about 2 weeks (5, 6), characterized by an increase in fibroblast numbers and accelerated collagen metabolism (7, 8).

Components of the extracellular matrix have been determined in blood of patients with fibrotic lung diseases, but in contrast to e. g. liver fibrosis there seems to be only a weak association between the concentrations of such analytes in blood and the activity and/or grade of the fibrosing process in the lung (9).

More promising results were obtained with measurements of extracellular matrix components in bronchoalveolar fluid from patients with interstitial lung diseases, e. g. sarcoidosis or farmer's lung. High concentrations of hyaluronan (hyaluronic acid), a glycosaminoglycan polymer and main constituent of loose connective tissue (10) and of the aminoterminal propeptide of type III procollagen (11), a split product originating from the synthesis and maturation of type III collagen, have been found in the lavage fluid (12, 13) and were proposed as indicators for assessing disease severity and outcome. Recently, *Hällgren* et al. (14) have reported elevated concentrations of hyaluronan in bronchoalveolar lavage fluid from patients with adult respiratory distress syndrome. They further found strongly increased concentrations of hyaluronan and aminoterminal propeptide of type III procollagen in the serum of these patients. We have made similar observations with regard to aminoterminal propeptide of type III procollagen and laminin in a preliminary study (15). As an alternative to conventional respiratory treatment of adult respiratory distress syndrome, the recently established concept of extracorporeal CO_2 removal by membrane-lungs in conjunction with apneic oxygenation (16–19) has been introduced. In the present work we have investigated the time course of the serum levels of hyaluronan, aminoterminal propeptide of type III procollagen, and laminin, a high molecular mass glycoprotein and main constituent of basement membranes (20), in sera of patients with severe adult respiratory distress syndrome treated with extracorporeal CO_2 removal.

Materials and Methods

Patients

Sixteen adult respiratory distress syndrome patients, mean age 29 (range 6–59) years, 10 females, were included in the study. All patients were admitted to the intensive care unit of the department of anaesthesia of our hospital within a total period of 1.5 years because of severe pulmonary failure and treated here with the method of extracorporeal CO_2 removal. The mean duration of conventional respiratory treatment before extracorporeal CO_2 removal was 15 ± 8 (mean $\pm$ SD) days. The mean duration of extracorporeal CO_2 removal treatment was 11 ± 6 days. Only patients with stage 4 (final stage) of the disease according to the classification scheme of *Morel* (21) were included. The following criteria had to be fulfilled for inclusion:

(i) demonstration of typical interstitial and alveolar infiltrates by X-ray and less than 20% of normal aerated lung regions by computer tomography,

(ii) mean pulmonary artery pressure of at least 35 mmHg,

(iii) alveolo-arterial oxygen pressure difference under 100% oxygen in excess of 525 mmHg, and

(iv) total compliance of the respiratory system less than 30 ml/cm H_2O.

The initiating events of adult respiratory distress syndrome and other pertinent information about our patients are given in table 1. Patients were classified as responders (n = 10, patients whose condition could be improved by extracorporeal CO_2 removal to such an extent that they could be weaned from the respirator) and non-responders (n = 6, non-survivors) to extracorporeal CO_2 removal as assessed by extravascular lung water volume, lung compliance and alveolo-arterial oxygen pressure difference. Two of the responders died, however, as a consequence of their primary injury (cases 2 and 4 in tab. 1). All patients received a continuous heparin application of 800–2500 U/h during the extracorporeal CO_2 elimination.

Extracorporeal CO_2 removal

The methodology of extracorporeal CO_2 removal is described in detail elsewhere (22–24).

Tab. 1. Summary of patient information.
NS: non-survivor, S: survivor, NR: non-responder, R: responder

Patient No.	Age	Gender	Underlying disease	Course	Class	Duration of artificial ventilation before extracorporeal CO_2 removal	Duration of extracorporeal CO_2 removal treatment
1	26	♀	Aspiration	NS	NR	19	12
2	6	♀	Polytrauma	NS	R	11	22
3	27	♂	Polytrauma	NS	NR	9	5
4	56	♀	Pneumonia	NS	R	11	5
5	20	♀	Polytrauma	NS	NR	16	1
6	30	♀	Aspiration	S	R	10	10
7	34	♂	Polytrauma	S	R	24	9
8	37	♀	Polytrauma	S	R	10	6
9	55	♀	Postoperative	S	R	11	20
10	46	♂	Pneumonia	S	R	15	21
11	59	♀	Postoperative	NS	NR	8	8
12	11	♂	Pneumonia	S	R	28	3
13	44	♀	Peritonitis	NS	NR	38	12
14	42	♀	Postoperative	S	R	20	14
15	16	♂	Polytrauma	S	R	9	15
16	45	♂	Aspiration	NS	NR	10	13

Quantities of pulmonary function and haemodynamics

On each day of extracorporeal CO_2 removal treatment the pulmonary function was assessed by a set of representative parameters. The intrapulmonal right-left shunt (Q_S/Q_T), alveolo-arterial oxygen pressure difference, mean pulmonary arterial pressure, and cardiac output were determined by standard techniques. Extravascular lung water was measured according to a double indicator method (25).

Specimen collection

Blood samples were collected immediately before and throughout the extracorporeal CO_2 removal treatment once a day. Blood was drawn from the inlet line of the membrane lungs, cooled on ice, clotted at room temperature for at least 60 minutes, and centrifuged. The serum was stored in aliquots at −70 °C until analysis, which was performed within one run for all analytes. We found no significant difference in the concentrations of extracellular matrix components between samples drawn at the membrane inlet line and after passage through the membrane lungs, or between samples drawn immediately before and after renewal of the membranes. While this does not exclude small long-term adsorption effects at the membranes, such effects, if they exist, should not bias our results and their interpretation because they will operate equally on all measurements.

Analytical methods

Laminin was measured in serum using a competitive RIA (Hoechst AG, Frankfurt, F. R. G.) with antibodies directed against antigenic determinants of fragment P1 (26). The analytical details have been described before (27). The within-run and day-to-day imprecisions were 3.1 and 4.9%, respectively ($\bar{x}$ = 1.8 kU/l). The concentrations of laminin in serum are given in arbitrary units based on the medium value of the serum concentrations in a healthy control population (26).

Hyaluronan was determined by a previously described radiometric assay (Pharmacia, Uppsala, Sweden) (28). The intra- and inter-assay coefficients of variation (CV) ($\bar{x}$ = 182 μg/l) were 6.9% and 7.3%, respectively.

The aminoterminal propeptide of type III procollagen was measured in various dilutions of serum using a radioimmunoassay (Behringwerke, Marburg, F. R. G.) based on intact polyclonal antibodies recognizing mainly the higher molecular mass (intact) propeptide Col 1-3 (29, 30). The within-run CV was 4.9% ($\bar{x}$ = 12 μg/l; n = 14), the day-to-day CV was 7.2% ($\bar{x}$ = 5.0 μg/l; n = 12).

Additional clinical chemical parameters, including alanine aminotransferase, creatinine, and haematocrit were determined according to standard methods (31).

Data analysis

For descriptive statistical analysis the daily mean concentrations of the respective analytes were computed separately for the survivor/non-survivor and responder/non-responder groups, respectively.

The association between parameters was tested using *Spearman*'s rank correlation coefficients (32). For testing differences between groups, the values of all numerical variables were first converted into ranks. These ranks, taken separately within each group, were subsequently used as input for an analysis of variance (ANOVA) procedure. By using ranks instead of the original values of a variable the ANOVA becomes equivalent to a non-parametric method, i. e. *Kruskal-Wallis*. Because of the relatively small number of cases on single days, the time-dependent quantities were computed by combining values obtained on single days into three groups: days 0−2, days 3−7, and day 8 and following. This stratification was chosen to cover the early, medium and late stages of the treatment process. The results of the statistical analysis were insensitive to small changes (± 1 day) of the temporal groupings.

All statistical computations were performed using the SAS PC V 6.03 statistical package (SAS Institute, Cary, NC).

Results

Time course of the serum concentrations of connective tissue components

The individual, time-dependent changes of the serum concentrations of aminoterminal propeptide of type III procollagen, laminin, and hyaluronan within the first 5−11 days after onset of the extracorporeal CO_2 removal treatment (day 0) are depicted in figure 1. The values of one patient who died within one day and of another patient whose treatment was stopped at day 3 were omitted from the figure. The initial serum concentrations of the connective tissue components were clearly above the reference limit in most patients. This was most pronounced for aminoterminal propeptide of type III procollagen (reference range: 2−12 μg/l (11)) and laminin (reference range: 1.08−1.90 kU/l (33)) and was seen in fewer cases for hyaluronan (reference range: 10−100 μg/l (34)). The patterns of values in responders who died did not differ distinctly from those of surviving responders. During the course of the treatment the laminin concentrations generally strongly decreased in both the responder and the non-responder group. The decrease was more pronounced in the responder than in the non-responder group (fig. 1). In one non-responder a slight increase in the concentration was observed. (For technical and organizational reasons laminin could not be determined in 4 patients).

The initial aminoterminal propeptide of type III procollagen concentrations in the responder group decreased with one exception. However, all values were still above the upper reference limit after 10 days. In the non-responder group, with one exception, the serum concentrations increased further after day 0 of extracorporeal CO_2 removal treatment.

Two of the responders and two of the non-responders to the extracorporeal CO_2 removal treatment had normal serum concentrations of hyaluronan at day 0, but clearly different trends with time of treatment were observed for this quantity in the two groups. In responders the serum levels declined or remained constant, while in the non-responder group all patients showed strongly incresing concentrations of serum hyaluronan.

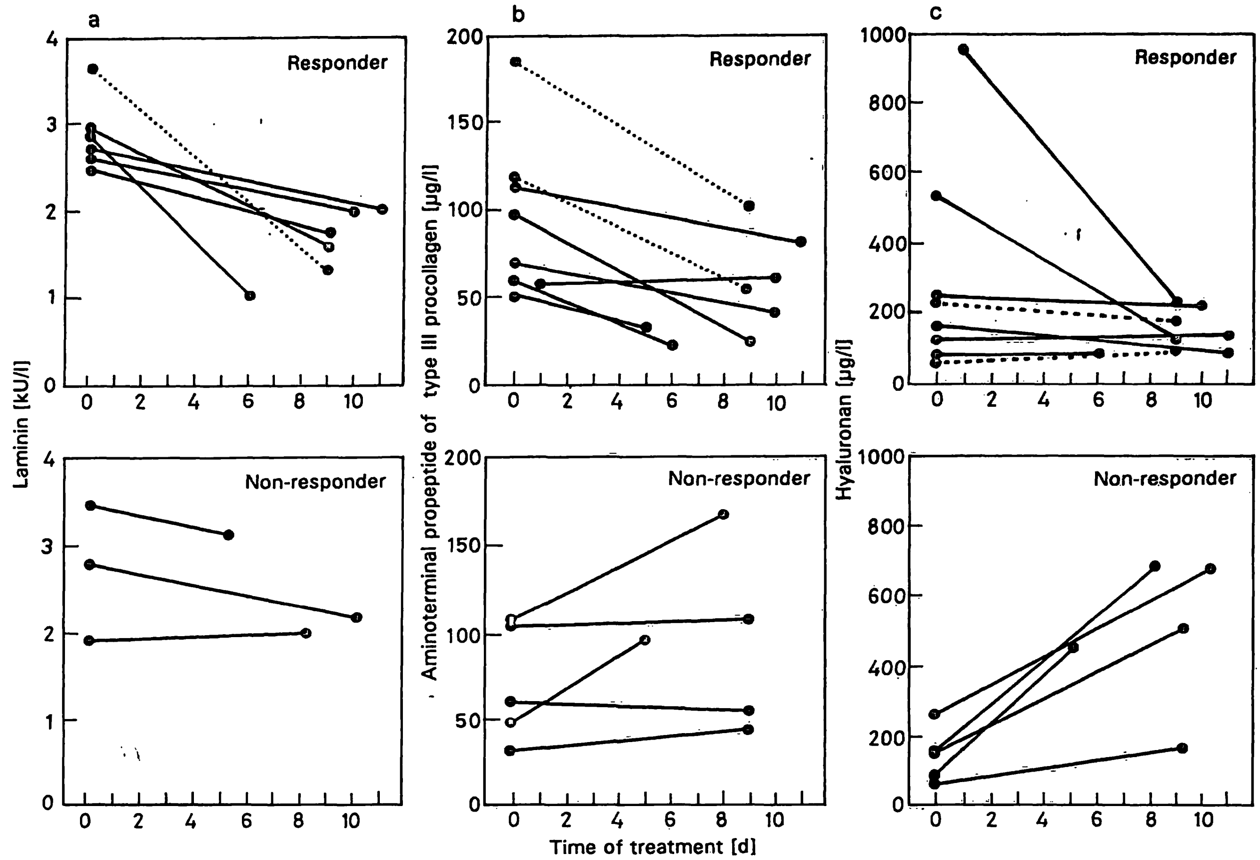

Fig. 1. Individual trends in the concentrations of the extracellular matrix components laminin (a), aminoterminal propeptide of type III procollagen (b), and hyaluronan (c) in serum of adult respiratory distress syndrome patients after start of extracorporeal CO_2 removal treatment. Values of non-surviving responders are shown as dotted lines.

The discrimination between responders and non-responders on the basis of connective tissue components in serum is expressed more clearly by the trends of the parameters than by their absolute values. To demonstrate these trends, figures 2 and 3 show for hyaluronan and aminoterminal propeptide of type III procollagen all changes relative to the initial values on day 0 for each patient. From these presentations it becomes obvious that within three days after start of the treatment all non-responders are fully separated

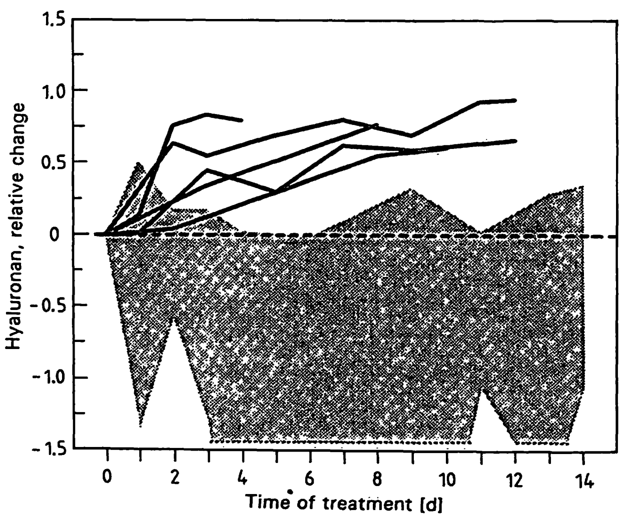

Fig. 2. Relative changes (concentration at time t — concentration at time 0 / concentration at time t) of individual serum concentrations of hyaluronan in non-responders (solid lines) as compared to their concentration at start of extracorporeal CO_2 removal treatment in comparison to the concentration range (shaded area) found for responders.

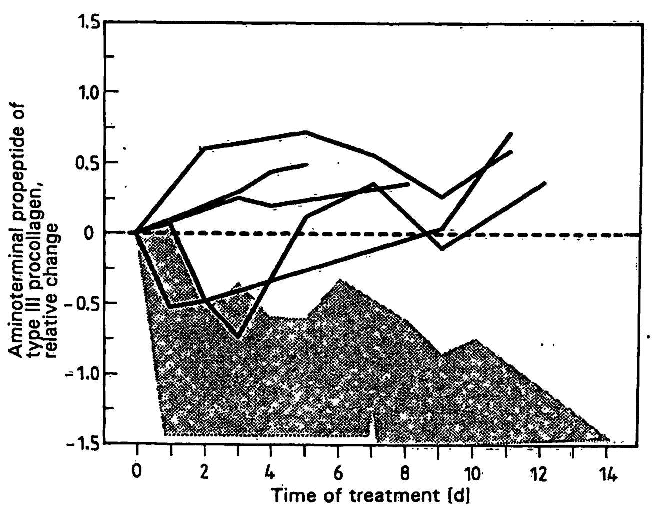

Fig. 3. Relative changes (concentration at time t — concentration of time 0 / concentration at time t) of individual serum concentrations of aminoterminal propeptide of type III procollagen in non-responders (solid lines) as compared to their concentration at start of extracorporeal CO_2 removal treatment in comparison of the concentration range (shaded area) found for responders.

from the responders by their increasing concentrations of serum hyaluronan and aminoterminal propeptide of type III procollagen.

Statistical analysis

No conspicuous correlations emerged between the concentrations of extracellular matrix components in serum and the physical quantities of lung function within individual patients. This lack of significance is partly caused by the small number of values for each patient and by the different trends of the physical and chemical parameters with time. For example alveolo-arterial oxygen pressure difference (fig. 4) decreased, as expected, in responders but remained high in non-responders, whereas the concentrations of the extracellular matrix components generally increased in non-responders during the course of the disease.

For testing differences between groups the original values of the variables were transformed into ranks as described under Data analysis. In the ANOVA procedure, 'time' (levels: days 0–2, days 3–7, and days 8 and following after start of extracorporeal CO_2 removal) and 'response' (levels: responder and non-responder) were used as main effects (tab. 2). Additionally, a second order combined effect of time-response was tested. With laminin, and to a lesser extent with creatinine, significantly different levels with regard to time were found, which can be explained (see fig. 1) by the fact that all patients showed decreasing serum laminin concentrations with time, whereas for the other quantities both decreasing and increasing values were found due to the classification of the patients in the responder and non-responder group, respectively. Significant differences between responders and non-responders were found in the serum concentrations of extracellular matrix components, serum creatinine, haematocrit, and in the alveolo-arterial oxygen pressure difference and shunt fraction. The high significances found for alveolo-arterial oxygen pressure difference and the shunt fraction are not surprising (and should not be considered as an independent effect) because these quantities were used as primary criteria for the establishment of the classification scheme. All three extracellular matrix components, but none of all other investigated quantities, were significantly different with regard to the second-order time-response effect. The ANOVA results obtained with rank data are further supported by the mean values of the different groups as shown in table 3.

Tab. 2. ANOVA results for the extracellular matrix components, some standard clinical chemical analytes and quantities of lung function.
(*: P < 0.05, **: p < 0.01, ***: p < 0.001, NS not significant)

Parameter	Effects		
	Time	Response	Time × Response
Hyaluronan	NS	**	*
Laminin	**	**	*
Aminoterminal propeptide of type III procollagen	NS	**	*
Alveolo-arterial oxygen pressure difference	NS	***	NS
Extravascular lung water	NS	NS	NS
Shunt fraction	NS	***	NS
Creatinine	*	***	NS
Alanine aminotransferase	NS	NS	NS
Haematocrit	NS	***	NS

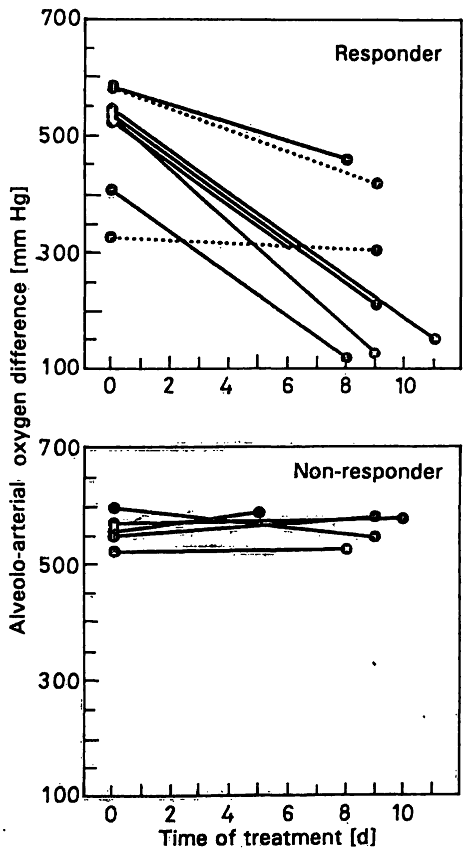

Fig. 4. Individual trends in the alveolo-arterial oxygen difference of adult respiratory distress syndrome patients after start of extracorporeal CO_2 removal treatment. Values of non-surviving responders are shown as dotted lines.

Discussion

By serial determination of the serum concentrations of aminoterminal propeptide of type III procollagen and hyaluronan in adult respiratory distress syndrome patients undergoing extracorporeal CO_2 removal treatment a prognostic evaluation of potential responders and non-responders can be reached as early

Tab. 3. Mean serum concentrations of connective tissue components, of some standard clinical chemical analytes and of lung function quantities according to time after onset of extracorporeal CO_2 removal treatment in responders (R) and non-responders (NR). The standard error of means is given in parenthesis below the values.

Parameter	Units	Day 0−2		Day 3−7		Day > 7	
		R	NR	R	NR	R	NR
Laminin	kU/l	2.77 (0.16)	2.72 (0.28)	1.75 (0.11)	2.35 (0.25)	1.69 (0.05)	2.19 (0.17)
Aminoterminal propeptide of type III procollagen	μg/l	85 (10)	92 (24)	45 (5)	93 (9)	56 (7)	130 (41)
Hyaluronan	μg/l	272 (68)	180 (41)	181 (41)	368 (68)	205 (43)	930 (311)
Creatinine	μmol/l	80 (6)	107 (11)	67 (3)	146 (20)	52 (3)	95 (12)
Alanine aminotransferase	U/l	25 (5)	56 (25)	24 (2)	34 (13)	31 (4)	46 (14)
Haematocrit	l/l	0.39 (0.005)	0.41 (0.013)	0.39 (0.005)	0.40 (0.006)	0.38 (0.005)	0.40 (0.011)
Alveolo-arterial oxygen pressure difference	mmHg	457 (20)	548 (13)	314 (26)	556 (12)	312 (25)	554 (9)
Extravascular lung water	l	1.19 (0.12)	1.38 (0.23)	0.98 (0.08)	1.29 (0.09)	0.73 (0.10)	0.84 (0.04)
Shunt fraction	%	34.6 (15)	43.4 (2.6)	23.4 (2.0)	51.3 (3)	23.3 (2.1)	44.1 (3.5)

as three days after onset of therapy. After start of the treatment all non-responders could be identified by their increasing serum concentrations of aminoterminal propeptide of type III procollagen and hyaluronan, whereas generally decreasing concentrations of these analytes were found in responders (figs. 2, 3).

In contrast to other respiratory diseases (9) strongly elevated concentrations of connective tissue components were noticed in sera of adult respiratory distress syndrome patients. This is in agreement with our previous observation (15) and with that of *Hällgren* et al. (14), who assessed patients early in the course of developing adult respiratory distress syndrome. Our study was focussed, however, on those patients with late stages of severe adult respiratory distress syndrome (cf. tab. 1, duration of artificial ventilation before extracorporeal CO_2 removal) subjected to extracorporeal CO_2 removal treatment (19). With regard to the statistical analysis, the serum concentrations of matrix components were the only quantities affected simultaneously by the effects response and time. This unique behaviour can be interpreted as the statistical confirmation of the trends visualized in figure 1.

Presently no clear explanation of the pathobiochemical basis of the significant changes in the concentrations of extracellular matrix components in serum during extracorporeal CO_2 removal can be given. One has to keep in mind that the serum levels of the matrix components are determined not only by the rate of production in tissues but also by secretion into the circulation, distribution and clearance (35). Aminoterminal propeptide of type III procollagen (36), hyaluronan (37) and laminin (38) are degraded in the liver by endothelial cells. Hyaluronan and smaller fragments of aminoterminal propeptide of type III procollagen are extracted by the kidneys (39). Theoretically all these pathways might contribute to the elevated levels observed here. We have no indication, however, that the distribution volume and the clearance rate are changed in our patients, as seen from the normal or only slightly elevated levels of alanine aminotransferase, creatinine and haematocrit (tab. 3).

Several experimental studies provide a solid basis for the assumption of enhanced production of hyaluronan and collagens in damaged lungs due to macrophage-driven fibroblast activation (40−45). An increased outflow into the alveolar space and into the lymph and blood circulation might explain the findings with respect to bronchoalveolar lavage as well as our observations with regard to the serum levels. It has been proposed that artificial ventilation might induce a mobilization of hyaluronan from the interstitial space by increasing the microvascular pressure (14). At least for the late stages of adult respiratory distress syndrome this hypothesis is not supported by our data: Although the lungs are resting during extracorporeal CO_2 removal and there is only low frequency positive pressure ventilation (which may not necessarily increase the transmural microvascular pressure), strongly increasing serum concentrations of hyaluronan were found in most non-responders. Only for laminin, which shows decreasing serum levels in all patients, are our data consistent with a contribution from artificial ventilation, which in the case might have influenced an increased turnover of alveolar basement membranes.

From animal experimental studies it was concluded that the volume of extravascular lung water is related to the amount of interstitial hyaluronan (46, 47). It has been hypothesized that the excessive accumulation of hyaluronan in the alveolar interstitial space makes a major contribution to interstitial oedema, due to its ability to immobilize water. If one assumes that the serum concentration of hyaluronan is related to its content in lung tissue, a significant correlation between hyaluronan and the extravascular lung water volume might be expected. Since we did not find consistent significant correlations in the majority of our patients the data do not support this hypothesis.

A principal difficulty in the interpretation of the results lies in the fact that adult respiratory distress syndrome in many cases is accompanied by severe primary injuries of various aetiologies. It seems impossible to differentiate fully between those effects specific for adult respiratory distress syndrome and those related to the polytraumatic lesions which led to the development of adult respiratory distress syndrome. Some arguments can be stated, however, for the existence of a more or less direct relation between adult respiratory distress syndrome and the connective tissue components in serum:

- Our patients were in late stages of severe adult respiratory distress syndrome with a mean pretreatment time of 15 ± 8 days at the start of extracorporeal CO_2 removal. The underlying disease had thus in most cases already been overcome.

- From figure 1 it can be seen that the serum concentrations of the connective tissue parameters of non-surviving responders are not distinct from those of the surviving responders.

Measurements of extracellular matrix components in serum present a clearly different approach to the assessment of severe adult respiratory distress syndrome, which otherwise depends on the measurement of respiratory function. Our results show that the successful outcome of extracorporeal CO_2 elimination in patients with adult respiratory distress syndrome is closely and early reflected by the time course of the serum concentrations of extracellular matrix components, aminoterminal propeptide of type III procollagen and hyaluronan, which suggests that these quantities might gain prognostic significance for extracorporeal CO_2 removal treatment in addition to gas exchange. Conceivably these non-invasive examinations might prove helpful in monitoring patients subjected to conventional therapy and in selecting different therapeutic options, including extracorporeal CO_2 removal.

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